ISOLATION OF TEXTILE DYE DEGRADING MICROORGANISMS FROM JAIPUR DISTRICT AND EVALUATION OF THEIR DEGRADATION POTENTIAL

Research Protocol

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Submitted by

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INTRODUCTION:

Environmental pollution has been recognized as one of the major hazard of the modern world. Due to rapid industrialization, lot of chemicals including dyes were manufactured and used in day to day life (Moorthi *et al.,* 2007).

Dyes usually have a synthetic origin and complex aromatic molecular structures which make them more stable and more difficult to biodegrade (Aksu, 2005). Approximately 10,000 different dyes and pigments are used industrially and over 0.7 million tons of synthetic dyes are produced annually worldwide. Due to modern life style, increasing urbanization and development the use of dyes and dye based products grows. Once they enter an ecosystem, dye-based pollutants initiate a series of processes, affecting both its biotic and abiotic elements (Małachowska *et al.,* 1997)**.**

Dyes are classified according to their application and chemical structure, and are composed of a group of atoms known as chromophores, responsible for the dye colour (Fu and Viraraghavan; 2001)**.** The three most common groups of dyes are azo, anthraquinone and phthalocyanine (Axelsson *et al.,* 2006), most of which are toxic and harmful for the environment. Disposal of these dyes into the environment causes serious damage, since they may significantly affect the photosynthetic activity of hydrophytes by reducing light penetration and also toxic to aquatic organisms due to their break down products (Hao *et al.,* 2000; Aksu *et al.,* 2005). One of the most pressing environmental problems related to dye effluents is the improper disposal of waste water from dyeing industry (Jayarajan *et al.,* 2011). Dyes produce an unacceptable intense coloring of water and soil bodies. They also decreased gas solubility in water bodies and block the passage of light to the lower depth of aquatic system. So they affect the photosynthesis which results in death of aquatic life causing foul smelling toxic water. Soil and water pollution through these pollutants poses a large environmental threat. Contamination of soil with dyes deteriorates change its biochemical and physicochemical properties it also limits the growth and development of plants. These compounds are largely responsible for changed fertility of soil (Tyczkowski, 1993).

Due to low biodegradability of dyes conventional effluent treatments systems are insufficient in treating waste water & soil. It is usually treated by physical or chemical

treatment process. These include physical or chemical flocculation, flotation, electro-flotation, membrane-filtration, electro kinetic–coagulation, electro chemical-destruction, and precipitation. These conventional chemical and physical methods are versatile and useful, but they only transfer the pollutant from one form to another, and produced secondary waste products. But these types of technologies are inefficient, expensive and have low adaptability to diverse type of dye effluent. Traditional methods for the cleanup of dyes in the textile waste water usually involve the removal of unwanted materials through sedimentation, filtration and chemical treatments such as flocculation, neutralization and electro-dialysis before disposal. These processes may not guarantee the treatment of toxic dye in the effluent. Moreover, considering the volume of wastes released during the industrial production process these are often laborious and expensive (Gopi *et al.,* 2012). Over the past decades, biological decolorization has been investigated as a method to transform, degrade or mineralize dyes (Banat *et al.,* 1996). Moreover, such decolorization and degradation is an environmental friendly and cost-competitive alternative to chemical decomposition processes (Verma *et al.,* 2003) .Bioremediation a economic viable safe and less expensive than conventional method of treatment (Chen *et al.,* 1999; Kanaly, Harayama, 2000; Watanabe 2001; Whyte *et al.,* 1997). Microorganisms can be applied in biodegradation at different environmental conditions (Margesin, Schinner 2001; Mishra *et al.,* 2001). Bioremediation is an option that offers the possibility to destroy or render harmless various contaminants using natural biological activity (Gupta and Mahapatra, 2003).

PART LITERATURE REVIEW:

The largest amount of dyes is used for dyeing of textile and it had been estimated that about 10% of the dye stuff used during these dying process does not bind to the fiber and is therefore released in to the sewage treatment system or the environment (Anliker, 1979; Chudgar, 1985; Reisch, 1996; Zollinger, 1991). These dyes pollute the environment and found to have adverse effect on soil and water. Waste water and soil samples from contaminated (irrigated with effluent water) and uncontaminated areas (not irrigated with effluent water) at 0-25cm depth from Bhairavgarh area Ujjain city were analyzed and found to be alkaline in nature. Chloride was highest (700mg/l) in W1

samples and lowest (500mg/l) in W2 samples while other parameters also found to have great variability. Soil samples were found to be neutral to slight alkaline in nature. A wide variation in cation and anion concentration, organic carbon and organic matter was also observed in contaminated soil samples as compared to uncontaminated samples (Ahmad *et al.,* 2012) The physico - chemical properties of soil of agricultural region and the water used for irrigation in Sanganer area of Jaipur were analyzed. Great variations were observed in the physico - chemical characteristics of the textile effluent contaminated soil samples when analysed by standard methods. The pH of the samples was found to be alkaline in nature (7.8 – 9.4). The values of electrical conductivity ranged from 0.75 - 1.15 mmhos/cm. The amount of % organic matter and % organic carbon ranged from 0.41 - 0.72%and 0.24 - 0.42 % respectively. Higher amount of heavy metals exceeding permissible limits were also present in soil (Jaishree and Khan 2014).

Several microorganisms were found to have the dye degradation potential. *Phanerochaete chrysosporium* is the most widely studied white-rot fungus, in this regard (Reddy, 1995). Bacterial isolates from soil and sludge sample belonging to *Bacillus sp*. *Alcaligenes sp*. and *Aeromonas sp*. were found to have high dye decolorization ability (Sharma *et al.,* 2004).The dye decolorizing isolates, *Bacillus sp., Klebsiella sp*. *Salmonella sp*. and *Pseudomonas sp.* were isolated from the textile effluent samples collected from Elampillai, Tamil Nadu (Ponraj *et al.,* 2011). In another study six bacterial strains viz., two species of *Bacillus*, two species of *Klebsiella*. one species each of *Planococcus* and *Micrococcus luteus* were isolated (Mohan *et al.,* 2013). Bacterial isolate SpNB6 was isolated from Environmental Treatment Plant Naroda G.I.D.C., Ahmedabad, Gujarat, India and was identified as *Pseudomonas otitidis* by 16s rDNA technology (Murty *et al.,* 2012). On the basis of 16s rRNA sequencing, two of the bacterial isolates in a microbial consortium C15 were found to belong to the *Chryseobacterium* genus and *Flavobacterium* genus (Khadijah *et al.,* 2009). Three bacterial isolates exhibiting strong decolourizing activity after 7 days of incubation with little deviation were identified up to species as *Micrococcus luteus*, *Listeria denitrificans* and *Nocardia atlantica* (Hassan *et al.,* 2013).

Cynobacteria like *Gloeocapsa pleurocapsoides* and *Phormidium ceylanicum* decolorized Acid Red 97 and FF sky Blue dye more than 80% after 26 days (Parikh, Madamwar, 2005). Decolorization of Direct yellow and Erio red dyes by bacterial and *actinomycetes* were studied by Waffa and Moawad (Moawad *et al.,* 2003). Decolourization of C.I Reactive Red 11 (mono azo) and C.I Reactive Yellow 84 (di azo) dyes was studied by developing bacterial consortia isolated from dye contaminated soil. Maximum decolourization of 85% was achieved at 100 mg /L of C.I Reactive Red 11 and 83% at 100 mg /L of C.I Reactive Yellow 84 after 72 h under shaking condition using 40% inoculum size of the respective consortia (Tandon *et al.,* 2014). Maximum dye decolorization of 89% was shown by *Pseudomonas sp.* and *Bacillus sp*. at the end of 144h under optimum condition. But the *Bacillus sp.* was found to be more efficient in dye decolorization (Ponraj *et al.,* 2011). Efficient decolorization of Remazol Brilliant Blue R (RBBR) was screened by using strains of terrestrial white rot (WRF) and marine fungi (MF). *Irpex lacteus* and *Pleurotus ostreatus (*WRF) were efficient dye degraders whereas were less efficient and selective degradations were observed with *Dactylospora haliotrepha* and *Aspergillus ustus* (MF). Seawater salinity often reduced decolorization efficiency of WRF but increased decolorization ability of MF. I. *lacteus* was found to remove 77% of RBBR in soil used at 150 µg/g within 6 weeks (Novotný et al., 2003). Bacterial consortia isolated from dye contaminated soil showed maximum decolourization of 85% at 100 mg /L of C.I Reactive Red 11 and 83% at 100 mg /L of C.I Reactive Yellow 84 after 72 h under shaking condition using 40% inoculum size of the respective consortia. Optimum pH and temperature for decolourization of each dye was 7.0 and 28°C ± 2°C, respectively. The rate of decolourization was enhanced to 89% for C.I Reactive Red 11 and 86% for C.I Reactive Yellow 84 in presence of additional carbon source and 86% for C.I Reactive Red 11 and 85% for C.I Reactive Yellow 84 in presence of additional nitrogen source. However, on addition of carbon and nitrogen sources together, the decolourization rate increased greatly to 94% for C.I Reactive Red 11 and 91% for C.I Reactive Yellow 84 (Tandon *et al.,* 2014). The enriched aerobic cultures of indigenous microbes were successfully used for decolorizing dye effluents. More than 50% of decolorization was achieved within 4 days of incubation. After 6 days of incubation, decolorization was achieved above 80%.

Maximum decolorization ability was exhibited by *Planococcus sp*. and *Bacillus sp.* at pH between 5-8 and temperature 37°C. Moreover, 10% (v/v) inoculums, glucose and peptone as carbon and nitrogen sources were found to be the optimum for decolorization. Both the isolates showed highest decolorization percentage of Coractive Blue 3R dye effectively (Mohan *et al.,* 2013). The strain SpNB6 showed complete decolorization of the dye RB 250 at 100 mg/L within 8 hr in static condition. The biodegradation was monitored by UV -Vis, HPTLC and FTIR analysis. Down Flow Fixed Film Batch Bioreactor gave good response up to 200 mg/L RB 250 dye concentration (Murty *et al.,* 2012). A microbial consortium labelled C15 showed good growth in agitation culture but the colour removal was best in static culture with 80 - 100% colour removed in less than 72 hours (Khadijah *et al.,* 2009). The bacterium *Micrococcus luteus* caused 60% decolourization of yellow FN2R and navy WB, and 85-90% of orange W3R, red FNR and blue FNR. Likewise, *Listeria denitrificans* decolourized 70-80% of Blue FNR, Orange W3R, Red FN R and Navy WB. In contrast, no significant decolourization of yellow FN2R was caused by the bacterium. *Nocardia atlantica* caused almost complete decolourization of Blue FNR and Red FNR, with at least 80% of other tested dyes (Hassan *et al.,* 2013).

STATEMENT OF THE RESEARCH PROBLEM:

"Isolation of Textile Dye Degrading Microorganisms from Jaipur District and Evaluation of their Degradation Potential"

Definition

Bioremediation: The use of biological organism to solve an environmental problem such as contaminated soil and water.

Decolorization: The act or process of depriving (removal) of color.

Biodegradation: Biodegradation is nature's way of recycling wastes, or breaking down organic matter into nutrients that can be used by other organisms.

OBJECTIVES OF THE STUDY:

The main objectives of present Research proposal are:-

- To study the physicochemical properties of contaminated and normal soil and water.
- To isolate dye degrading Microorganisms from contaminated soil and water.
- To characterize isolated microorganisms on the basis of morphological and biochemical characteristics.
- To determine the degradation potential of isolated microorganisms.
- To determine the decolorization potential of isolated microorganisms.

SCOPE OF THE STUDY:

Due to low biodegradability of dyes conventional effluent treatments systems are insufficient in treating waste water & soil. It is usually treated by physical or chemical treatment process. But these types of technologies are inefficient, expensive and have low adaptability to diverse type of dye effluent. Bioremediation is an economically viable, safe and less expensive technique than conventional treatment methods. Therefore in the present study biodegradation and decolorization potential of the microorganisms isolated from Sanganer and Bagru region of Jaipur city will be evaluated.

SIGNIFICANCE OF THE STUDY:

- Bioremediation is a natural process and is therefore perceived by the public opinion as an acceptable waste treatment process for contaminated material such as water and soil.
- Use of microorganism for the removal of contaminated soil and water will serve two purposes at the same time, one is low cost and other one is eco-friendly approach for degradation of dyes.
- Efficient dye degrading microorganisms for treatment of contaminated soil and water will be isolated.

LIMITATIONS:

- Due to time constraint the effect of isolated dye degrading microorganisms will not be studied in field conditions.

- Dye degrading potential of isolated microorganisms cannot be predicted for all type of dyes as different dyes contain different compounds with complex structures.
- Besides dye other pollutants are also present in contaminated samples which may interfere in decolorization process.
- Degradation of other pollutants if present in collected samples will not be studied in the present study.

METHODOLOGY:-

Collection of soil and waste water Samples: Samples will be collected from textile industries of Sanganer and Bagru region of Jaipur, Rajasthan. Samples will be collected in sterilized sealed polythene bags & plastic bottles and will be aseptically brought to the laboratory and kept at 4°C for further research.

Physicochemical analysis of collected samples:

Physicochemical properties (pH, conductivity, BOD, COD, caco3, potassium, organic matter and carbon etc.) will be determined by standard methods (UV-visible spectroscopy, Atomic absorption spectroscopy, titrimetric method.)

Isolation of microorganisms from collected samples:

Microorganisms will be isolated from the affected soil and water by using serial dilution, agar plating and enrichment culture technique.

Characterization and Identification of isolated microorganisms:

Isolated microorganisms will be characterized on the basis of morphological (cell shape, colony characteristics, staining, etc.) and biochemical (IMViC, catalase, amylase, carbon utilization, etc.) characteristics according to standard protocols

Screening of degradation & decolorization potential of isolated microorganisms:

Degradation and decolorization potential of isolated microorganisms will evaluated by photometric methods using (UV-VIS spectrophotometer) at various time interval.

MONTH WISE PLAN OF WORK:-

Duration	**Activity**
3 Months (December 2014- May 2016)	Literature survey and it will be in continuation till 22 months
3 Months (December 2014- Febaury 2015)	Collection of soil and water samples from textile industries.
2 Months (March 2015- April 2015)	Analysis of the physicochemical properties of contaminated soil and water
5 Months (May 2015- September 2015)	Isolation of dye degrading microorganisms from contaminated soil and water.
4 Months (October 2015-January 2016)	Characterize and identification isolated microorganisms on the basis of morphological and biochemical characteristics.
4 Months (Febuary 2016- May 2016)	Determination of biodegradation and decolorization potential of isolated microorganisms
3 Months (June 2016- August 2016)	Compilation and verification of the work, report/thesis writing
	Total Time (expected) = 24 Months

MONTH WISE PLAN OF WORK (GRAPHICAL REPRESENTATION)

Activity / Months	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
Literature survey																								
Collection of soil and water samples																								
Analysis of the physicochemical properties of contaminated soil and water																								
Isolation of dye degrading microorganisms																								
Characterization and identification microorganism																								
Determination of biodegradation and decolorization potential of microrganisms																								
Thesis preparation																								

REFERENCES:

1. Ahmad, M.T. Manderia, S. and Manderia, K. (2012) 'Influence of dye industrial effluent on physico chemical characteristics properties of soil at Bhairavgarh, Ujjain, MP, India, *Research Journal of Environment Sciences* , 1(1), pp. 50-53.
2. Aksu, Z. (2005) 'Application of biosorption for the removal of organic pollutants: A review', *Process Biochemistry,* 40(3/4), pp. 997-1026.
3. Aksu, Z. Kilic, N. Ertugrul, V. and Donmez, G. (2005) 'Inhibitory effects of chromium (VI) and Remazol black on chromium (VI) and dye stuff removals by *Trametesversicolor*', *Enzyme Microbial Technology,* 40(5), pp. 1167-1174.
4. Anliker, R. (1979) 'Exotoxicology of dyrstuffs. A join effort by industry Ecotox', Environment Safety, 3, pp. 59-74.
5. Axelsson, J. Nilsson, U. Terrazas, E. Aliaga, T.A. and Welander, U. (2006) 'Decolorization of the textile dyes reactive red 2 and reactive blue for using *Bjerekandera* sp. strain Bol 13 in a continuous rotating biological contactor reactor', *Enzyme Microbial Technology,* 39(1), pp. 32-37.
6. Banat, I.M. Nigam, P. Singh, D. and Marchant, R. (1996) 'Microbial decolorization of textile dye containing effluents: A review', *Bioresource Technolnology*, 58(3), 217-227.
7. Chen, W. Brhlmann, F. Richins, R.D. and Mulchandani, A. (1999) 'Engineering of improved microbes and enzymes for bioremediation', *Current Opinion Biotechnology,* 10(1), pp. 137-141.
8. Chudgar, R.J. (1985) 'Azo dyes In kirk-Othmer Encyclopedia of chemical Technology', (ed.), Press, Kroschwitz, wiley, New York, pp. 821-875.
9. Fu, Y. and Viraraghavan, T. (2001) 'Fungal decolorization of dye wastewaters: a review', *Bioresource Technology,* 79(3), pp. 251-262.
10. Gopi, V. Akhilesh, U. and Soundararajan, N. (2012) 'Bioremediation potential of individual and consortium non-adapted fungal strains on *Azo dye* containing textile effluent', *Advance Applied Science Res*earch, 3(1), pp. 303-311.
11. Gupta, R. and Mahapatra, H. (2003) 'Microbial biomass an economical alternative for removal of heavy metals from the waste water', *Indian journal of experimental biology,* 41(9), pp. 945-966.

12. Hao, O.J. Kim, H. Chaing, P.C. and Holt, J.G. (2000) 'Decolorization of wastewater critical reviews', *Environmental ScienceTechnology*, 30(4), pp. 449-505.
13. Hassan, M.M. Alam, M.Z. and Anwar, M.N. (2013) 'Biodegradation of Textile Azo Dyes by Bacteria Isolated from Dyeing Industry Effluent', *International Research Journal of Biological Scince,* 2(8), pp. 27-31.
14. Jaishree, and Khan, T.I. (2014) 'Physico-chemical analysis of contaminated soil collected from different areas of sanganer textile industries jaipur (rajasthan)', *International Journal of Scientific and Research Publications,* 4(3) pp. 1-4.
15. Jayarajan, M. Arunachalam, R. and Annadurai G. (2011) 'Agricultural wastes of jackfruit peel nano-pourous adsorbent for removal of rhodamine dye', *Asian Journal Applied Science,* 4(3), pp. 263-270.
16. Kanaly, R.A. and Harayama, S. (2000) 'Biodegradation of High-Molecular-Weight Polycyclic Aromatic Hydrocarbons by Bacteria', *Journal of Bacteriology*, 182(8), pp. 2059-2067.
17. Khadijah, O. Lee, K.K. and Mohd Faiz, F.Abdullah. (2009) 'Isolation, screening and development of local bacterial consortia with azo dyes decolourising capability', *Malaysian Journal of Microbiology*, 5(1), pp. 20-32.
18. Małachowska Jutsz, A. Mrozowska, J. Kozielska, M. and Miksch, K. (1997) 'Enzymatic activity of soil contaminated with petroleum products during the process of soil detoxication', *Biotechnologia*, 1, pp. 79–91.
19. Margesin, R. and Schinner, F. (2001) 'Potential of halotolerant and halophilic microorganisms for biotechnology', *Extremophiles*, 5(2), pp. 73–83
20. Mishra, S. Jyot, J. Kuhad, R.C. and Lal, B. (2001) 'Evaluation of Inoculum Addition To Stimulate In Situ Bioremediation of Oily-Sludge-Contaminated Soil', *Applied Environment Microbiology*, 67(4), pp. 1670-1675.
21. Mohan, V. Madhumitha, M. Menon, S. and Saranya, D.K. (2013) 'Isolation and Screening of potential Dye decolorizing bacteria from Textile dye effluents in Tamil Nadu, India', *Journal of Academia and Industrial Research (JAIR)*, 2(2), pp. 18-22.

22. Murty, S.D. Patel, S.D. Soni, R. and Bhatt, N. (2012) 'Isolation and Identification of Bacterial Culture for Azo Dye Degrading Capability', *International Journal of Research in Chemistry and Environment,* 2(4), pp. 69-79.
23. Moawad, H. Waffa, M. Abd Rahim, E.I. and Khalafallah, M. (2003) 'Evaluation of biotoxicity of textile dyes using two bioassays, *Journal of Basic Microbiology,* pp. 218-229.
24. Moorthi, P.S. Selvam, S. Sasikalaveni, A. Murugesan, K. and Kalaichelvan, P.T. (2007) 'Decolorization of textile dyes and their effluents using white rot fungi', *African Journal of Biotech.* 6(4), pp. 424-429.
25. Novotný, C. Rawal, B. Bhatt, M. Patel, V. Šašek, H.P. and Molitoris. (2003) 'The Utilization of Bioremediation to Reduce Soil Contamination: Problems and Solutions*, NATO Science Series,* 19(1), pp. 143-148.
26. Parikh, A. and Madamwar, D. (2005) 'Textile dye decolorization using cyanobacteria', *Biotechnol Lettrature,* 27(5), pp. 323-326.
27. Ponraj, R.M. Gokila, K. and Vasudeo, Z. (2011) 'Bacterial Decolorization Of Textile Dye-Orange', *International Journal of Advanced Biotechnology and Research,* 2(1), pp. 168-177
28. Reisch, M.S. (1996) 'Asian textile dye makers are a growing power in changing market', *Chemical & Engineering News,* 15, 10-12.
29. Reddy, C.A. (1995) 'The potential for white rot fungi in the treatment of pollutants', *Current Optional Biotechnology*, 6(1), pp. 320–328.
30. Sharma, D.K. Saini, H.S. Singh, H. Chimni, S.S. and Chada, B.S. (2004) 'Biological treatment of textile dye Acid violet-17 by bacterial consortium in an up-flow immobilized cell bioreactor', *Letters in Applied Microbiology*, 38(5), pp. 345-350.
31. Tandon Shalini, A. Sana, S. and Kumar, R. (2014) 'Development and use of soil bacterial consortia for bioremediation of dye polluted soil and municipal waste water**',** *Global Journal of Biosciences and Biotechnology*, 3(3), pp. 284-295.

32. Tyczkowski, A. (1993) 'Removal of petroleum contaminants of soil and groundwater physiochemical and biotech', *Enzyme Technology,* 3(1), pp. 10-13.
33. Verma, P. and Madamwar, D. (2003) 'Decolorization of synthetic dyes by a newly isolated strain of *Serratia marcescens*', *World Journal of Microbiology Biotechnology*, 19(6), pp. 615-618.
34. Watanabe, K. (2001) 'Microorganisms Relevant to Bioremediation', *Current Opinon Biotechnology,* 12(1), pp. 237-241.
35. Whyte, L.G. Bourbonnie`re, L. and Greer, C.W. (1997) 'Biodegradation of Petroleum Hydrocarbons by Psychrotrophic *Pseudomonas* Strains Possessing Both Alkane (alk) and Naphthalene (nah) Catabolic Pathways', *Applied Environment Microbiology,* 63(11), pp. 3714-3719.
36. Zollinger, H. (ed.) (1991) '*Color Chemistry: Syntheses, Properties and applications of organic Dyes and Pigments* . VCH, Weinheim.

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